



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L411

Nitrification Potential – Shaken Soil Slurry Method

Standard Operating Procedure

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1. Purpose

This procedure describes a rapid method for measuring the potential rate of ammonium oxidation in soils using a shaken soil slurry. The method provides an estimate of the activity of the existing ammonium-oxidizing microbial population at the time of sampling. It is based on ISO 15685 (*Soil quality – Determination of potential nitrification and inhibition of nitrification – Rapid test by ammonium oxidation*) (International Organization for Standardization, 2012).

Ammonium oxidation is the first step in autotrophic nitrification. Soil is incubated in a buffered (pH 7.2) ammonium sulfate solution with sodium chlorate added to inhibit nitrite oxidation. The accumulation of nitrite (NO_2^-) over a 6-hour incubation period serves as the measure of potential nitrification activity. Because the generation time of ammonia-oxidizing bacteria exceeds 10 hours, the assay measures the activity of the population present at sampling, not population growth.

This method can be used to compare soils (e.g., polluted vs. reference), to screen the effects of management practices or chemical substances on nitrifying communities, and to evaluate the impact of biosolids or waste materials on soil nitrification.

2. Scope

- **Applies to:** Field-moist soils containing a population of nitrifying microorganisms; assessment of nitrification inhibition by chemical substances (except volatiles with Henry's constant $H > 1$), biosolid extracts, and polluted soil mixtures
- **Does not apply to:** Soils with no detectable nitrifying population; measurement of in situ nitrification rates (this is a potential activity assay under optimized conditions); volatile inhibitors
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve experimental design, ensure training compliance
Lab Manager	Prepare reagents, maintain shaking incubator
Trained Personnel	Perform assay, record and report results
New Students	Complete training before independent use

4. Definitions

- **Ammonia oxidation:** The first step of nitrification; conversion of NH_4^+ to NO_2^- by ammonia-oxidizing bacteria (AOB) and archaea (AOA); the rate-limiting step of nitrification in most soils.
- **Autotrophic nitrification:** Nitrification carried out by chemolithoautotrophic microorganisms (AOB, AOA, and nitrite oxidizers) that use the energy from ammonia oxidation to fix CO_2 ; distinguished from heterotrophic nitrification.
- **Chlorate inhibition:** Addition of sodium chlorate (ClO_3^-) to suppress nitrite oxidation, allowing NO_2^- to accumulate and be measured as the product of ammonia oxidation; used in some nitrification potential assays.
- **Nitrite oxidation:** The second step of nitrification; conversion of NO_2^- to NO_3^- by nitrite-oxidizing bacteria (NOB); not rate-limiting under normal conditions.
- **Nitrification potential (NP):** The short-term rate of NO_3^- (or $\text{NO}_2^- + \text{NO}_3^-$) production in a soil slurry amended with excess NH_4^+ and incubated aerobically; reflects the size and activity of the extant nitrifier community; expressed as $\mu\text{g NO}_3\text{-N g}^{-1} \text{ soil h}^{-1}$.
- **Slurry method:** The standard technique for measuring nitrification potential; soil is suspended in a buffered ammonium sulfate solution at a defined soil-to-solution ratio and shaken aerobically at 25 °C; sequential subsamples are taken and analyzed for $\text{NO}_2^- + \text{NO}_3^-$.

5. Safety and Hazards

Hazards

- **Chemical:** Sodium chlorate (NaClO_3) is an oxidizer; avoid contact with organic materials and combustible substances. Potassium chloride (4 M KCl) is a mild irritant. Ammonium sulfate is a mild irritant.
- **Physical:** The orbital shaking incubator has moving parts; keep hair and loose clothing clear. Centrifuge operation poses standard mechanical hazards.

5.1. Required PPE

- ☐ Safety glasses
- ☐ Lab coat
- ☐ Nitrile gloves

5.2. Emergency Procedures

For chemical spills, refer to [SOP-L003 Hazardous Materials Handling](#). For sodium chlorate spills, do not use organic absorbents (sawdust, paper towels); use vermiculite or sand.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Orbital shaking incubator	Thermostatically controlled at 25 +/- 2 C; capable of 175 rpm
Erlenmeyer flasks (250 mL)	One per replicate
Centrifuge	Capable of 3,000 x g; see <i>SOP-L108</i> or <i>SOP-L109</i>
Analytical balance	0.001 g resolution; see SOP-L102
Volumetric flasks (100 mL, 1 L)	Class A
Graduated cylinders	10, 100, 1000 mL
Pipettes	2 mL (for slurry sampling) and assorted
Test tubes with caps	For subsamples
Filter funnels and filter paper	High-filtration-speed paper; chemical purity can be below the highest grade
pH meter	Calibrated per SOP-L300

6.2. Reagents

Reagent	Concentration / Preparation
Distilled or deionized water	From SOP-L11
Potassium dihydrogen phosphate (KH ₂ PO ₄)	0.2 mol/L solution
Dipotassium hydrogen phosphate (K ₂ HPO ₄)	0.2 mol/L solution
Sodium chlorate (NaClO ₃)	0.5 mol/L solution
Ammonium sulfate ((NH ₄) ₂ SO ₄)	Solid; 0.198 g per liter of test medium
Sodium bicarbonate (NaHCO ₃)	5 mmol/L solution (optional; see Section 6.2)
Potassium chloride (KCl)	4 mol/L solution (extraction/stop solution)

7. Procedure

7.1. Stock Solution A (Phosphate Buffer)

Combine the following in a volumetric flask and bring to 200 mL with distilled water:

- 28 mL of 0.2 mol/L KH₂PO₄
- 72 mL of 0.2 mol/L K₂HPO₄

This yields a phosphate buffer at approximately pH 7.2.

7.2. Test Medium

Prepare 1 L of test medium as follows:

1. Transfer 10 mL of Stock Solution A to a 1 L volumetric flask
2. Add 10–30 mL of 0.5 mol/L NaClO₃ (see note below on concentration selection)
3. Add 0.198 g of (NH₄)₂SO₄
4. If a carbon source is needed (e.g., when testing biosolids with high nitrate), add 10 mL of 5 mmol/L NaHCO₃
5. Dilute to 1 L with distilled water
6. Verify that the pH is approximately 7.2; adjust if necessary

The final test medium contains approximately 1 mmol/L potassium phosphate buffer, 5–15 mmol/L sodium chlorate, and 1.5 mmol/L ammonium sulfate.

Note

The sodium chlorate concentration should be sufficient to inhibit biological nitrite oxidation without negatively affecting ammonium oxidation. If the appropriate concentration for your soil type is unknown, run a preliminary trial at 5, 10, and 15 mmol/L NaClO₃ to identify the optimal level.

7.3. Preparing the 4 M KCl Stop Solution

Dissolve 298.2 g KCl in distilled water and bring to 1 L in a volumetric flask. This solution is used to halt ammonium oxidation at each sampling time.

7.4. Soil Moisture Determination

Determine the gravimetric moisture content of the soil per [SOP-L400](#) (dry at 105 C, 24 h). This value is needed to calculate the volume of water in the initial soil sample and to express results on a dry-mass basis.

7.5. Setting Up the Incubation

1. Weigh approximately 25 g of field-moist soil into each of at least three replicate 250 mL Erlenmeyer flasks
2. Calculate the volume of water already present in the soil:

$$V_{\text{water}} = M_{\text{soil}} \times \theta_g$$

where M_{soil} = mass of moist soil (g) and θ_g = gravimetric moisture content (g water / g dry soil).

3. Calculate the volume of test medium to add:

$$V_{\text{medium}} = V_{\text{total}} - V_{\text{water}}$$

where V_{total} = desired total liquid volume (typically 100 mL).

4. Add the calculated volume of test medium to each flask
5. Place the flasks upright on the orbital shaking incubator set to 25 +/- 2 C and 175 rpm (sufficient to keep solids suspended)

! Important

If testing chemical substances, dissolve them in the phosphate buffer and add to the test medium before diluting to 1 L. For substances with low water solubility, use mechanical dispersion or a low-toxicity vehicle (organic solvent, emulsifier). If a vehicle is used, include an additional control to assess its effect on ammonium oxidation.

7.6. Sampling the Slurry

1. At 2 h and 6 h after the start of incubation, shake each flask well to resuspend solids and withdraw a 2 mL aliquot of the slurry. Ensure the ratio of solution to soil is consistent across all samples.
2. Dispense the 2 mL aliquot into a test tube
3. Immediately add 2 mL of 4 M KCl to stop ammonium oxidation
4. Centrifuge at 3,000 x g for 2 minutes, or filter through high-speed filter paper
5. Transfer the clear supernatant or filtrate to a clean, labeled tube

7.7. Nitrite Analysis

Analyze the extracts for NO_2^- -N using a suitable colorimetric method (e.g., ISO 14256-2; diazotization with sulfanilamide and N-(1-naphthyl)ethylenediamine). Extracts may be stored at 4–8 C but must be analyzed within 24 hours.

8. Quality Control and Acceptance Criteria

8.1. Calculations

Potential ammonium oxidation rate:

$$\text{Rate} = \frac{[\text{NO}_2^- \text{-N}]_{6h} - [\text{NO}_2^- \text{-N}]_{2h}}{\Delta t \times M_{\text{dry}}} \times V_{\text{total}}$$

where the NO_2^- -N concentrations are in ug/mL (= mg/L), Δt = time interval (4 h), M_{dry} = dry mass of soil (g), and V_{total} = total liquid volume in the flask (mL). Report results as ng NO_2^- -N per g dry soil per hour.

Inhibition (%) by a test chemical:

$$\text{Inhibition (\%)} = \frac{R_{\text{control}} - R_{\text{treatment}}}{R_{\text{control}}} \times 100$$

where R = ammonium oxidation rate.

Toxicity assessment for polluted soil mixtures:

When mixing polluted and unpolluted (reference) soils, the polluted soil is considered toxic if:

$$A_m + s_m < 0.9 \times \frac{A_c + A_p}{2}$$

where A_m = mean activity in the mixture, s_m = standard deviation in the mixture, A_c = mean activity in the control soil, and A_p = mean activity in the polluted soil (each tested separately). This threshold applies with a minimum of four replicates.

8.2. QC Requirements

- Use a minimum of three replicates per treatment
- Four replicates are required when applying the toxicity assessment equation above
- Include a negative control (soil with no ammonium addition) and a positive control (reference soil with known nitrification activity) in each batch
- Verify that ammonium oxidation is linear between the 2 h and 6 h sampling points. If non-linearity is suspected, add sampling at 0 h and 4 h to characterize the time course.
- Blank controls (test medium without soil) should show no significant nitrite accumulation
- Report the coefficient of variation among replicates. A CV greater than 15% suggests sampling inconsistency or soil heterogeneity; consider additional replicates.

9. Waste Disposal

Waste Type	Disposal Method
Soil slurries (non-hazardous)	Drain liquid; dispose of soil in standard trash
KCl extracts	Drain with copious water
NaClO ₃ -containing solutions	Collect in oxidizer waste; EH&S pickup. Do not mix with organic waste.
Used filter paper and tubes	Standard trash (if non-hazardous soil)

10. References

International Organization for Standardization. (2012). *Soil quality – determination of potential nitrification and inhibition of nitrification – rapid test by ammonium oxidation* (ISO 15685:2012). International Organization for Standardization. <https://www.iso.org/standard/55080.html>

11. Training Record

Before performing the nitrification potential assay independently, personnel must:

1. Read this SOP in full
2. Complete [SOP-L002 Laboratory Safety](#) and [SOP-L003 Hazardous Materials Handling](#) training
3. Review the SDS for sodium chlorate
4. Complete [SOP-L400 Soil Moisture](#) training
5. Observe a trained operator perform the full assay from slurry setup through nitrite analysis
6. Perform the assay under supervision with acceptable replicate precision (CV less than 15%)
7. Be signed off by the PI or lab manager

12. Appendix A: Example Calculation

```
# Nitrification potential calculation per ISO 15685

# Input data
no2_2h_mg_L <- 0.15      # NO2-N at 2 h (mg/L in the extract)
no2_6h_mg_L <- 0.95      # NO2-N at 6 h (mg/L in the extract)
delta_t_h <- 4           # Time interval (h)
soil_moist_g <- 25.0      # Mass of field-moist soil (g)
theta_g <- 0.22          # Gravimetric moisture (g water / g dry soil)
V_total_mL <- 100        # Total liquid volume in the flask (mL)

# Dry mass of soil
M_dry_g <- soil_moist_g / (1 + theta_g)

# Potential ammonium oxidation rate (ng NO2-N / g dry soil / h)
# Convert mg/L to ug/mL (same units), then multiply by V_total to get
# total ug in the flask, divide by dry mass and time, convert to ng.
rate_ng_per_g_per_h <- ((no2_6h_mg_L - no2_2h_mg_L) *
                        V_total_mL / (delta_t_h * M_dry_g)) * 1000

cat(sprintf("Dry soil mass:           %.2f g\n", M_dry_g))
cat(sprintf("Delta NO2-N:           %.2f mg/L\n",
            no2_6h_mg_L - no2_2h_mg_L))
cat(sprintf("Nitrification potential:  %.1f ng NO2-N / g dry soil / h\n",
            rate_ng_per_g_per_h))
```

13. Appendix B: Reagent Preparation Quick Reference

Reagent	Preparation
0.2 mol/L KH_2PO_4	Dissolve 27.22 g in 1 L distilled water
0.2 mol/L K_2HPO_4	Dissolve 34.84 g in 1 L distilled water
Stock Solution A	28 mL KH_2PO_4 + 72 mL K_2HPO_4 + 100 mL water
0.5 mol/L NaClO_3	Dissolve 53.23 g in 1 L distilled water
4 mol/L KCl	Dissolve 298.2 g in 1 L distilled water
5 mmol/L NaHCO_3	Dissolve 0.420 g in 1 L distilled water
Test medium (1 L)	10 mL Stock A + 10–30 mL NaClO_3 + 0.198 g $(\text{NH}_4)_2\text{SO}_4$ + water to 1 L

14. Appendix C: Troubleshooting

Symptom	Probable Cause	Corrective Action
No nitrite accumulation	Soil has no active nitrifying population; NaClO_3 concentration too high	Test a reference soil in parallel; reduce NaClO_3 to 5 mmol/L
Non-linear NO_2^- accumulation	Nitrifier population growing during incubation; substrate depletion	Add intermediate time points (0 h, 4 h); use only the linear portion of the curve
High variability among replicates	Heterogeneous soil; inconsistent slurry sampling	Sieve and mix soil thoroughly; ensure slurry is well-shaken before each aliquot
Nitrite detected in controls (no NH_4^+)	Background nitrification from soil organic N	Note in results; subtract control values from treatment values
NaClO_3 appears to inhibit ammonium oxidation	Concentration too high for this soil	Run a NaClO_3 dose-response trial (5, 10, 15 mmol/L)